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SATIVANINE-K: AN ADDITIONAL *N*-FORMYL CYCLOPEPTIDE ALKALOID FROM *ZIZYPHUS SATIVA*

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Key Word Index—*Zizyphus sativa*; Rhamnaceae; sativanine-K; *N*-formyl cyclopeptide alkaloid.

Abstract—From the bark of *Zizyphus sativa* a 13-membered *N*-formyl cyclopeptide alkaloid containing a short side chain has been isolated for the first time. The structure was determined by spectroscopic methods and chemical degradation.

INTRODUCTION

Zizyphus species of the family Rhamnaceae are commonly available in tropical and subtropical countries where they are used in traditional medicine [1]. *Z. sativa* Gaertn is a 5–6 m tall tree native to the Hazara district of Pakistan and its bark is used to heal ulcers and wounds while its fruits are used in the treatment bronchitis [2]. Recently we reported the isolation of eight new cyclopeptide alkaloids from this plant [3–5]. Extensive chromatography of the crude bases furnished a further previously unknown alkaloid. Sativanine-K (1) is the first naturally occurring 13-membered *N*-formyl cyclopeptide alkaloid containing a short side chain. The structure of this new compound was determined mainly by mass spectrometry corroborated by other physical and chemical methods.

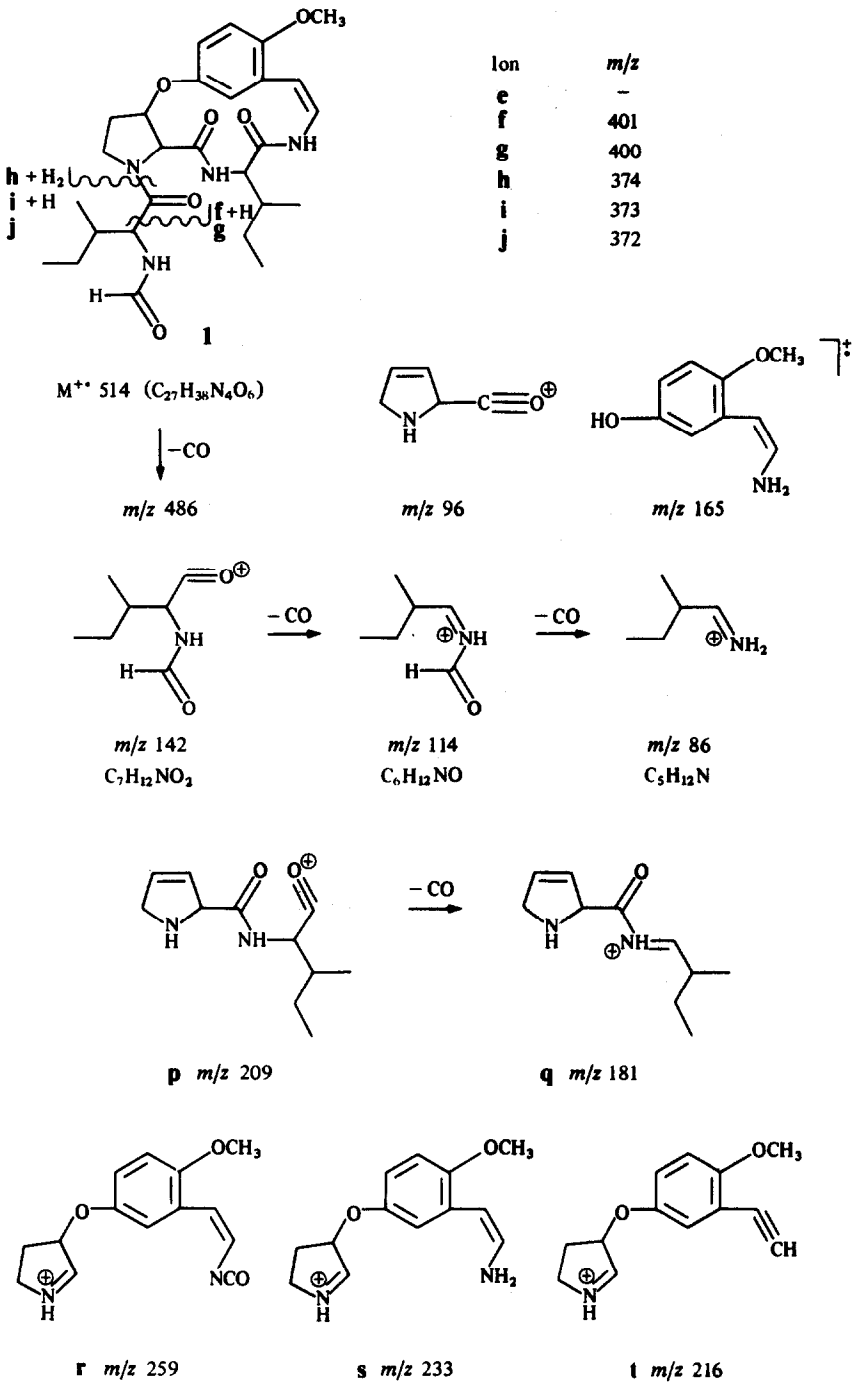
RESULTS AND DISCUSSION

The alkaloid (1) was isolated using consecutive TLC and chromatotron from fraction 12 of the CC. It gave a slight yellow colour with Dragendorff's reagent. The IR spectrum exhibited bands for –NH, –OMe, –NH–CO, >C=C< and Ar–O–C. The UV spectrum showed absorption maxima at 320 and 260 nm typical for a styrylamine chromophore in 13-membered cyclopeptide alkaloids [6]. The elementary composition of 1 was determined by high resolution mass spectrometry as $C_{27}H_{38}N_4O_6$. Its mass spectrum was most revealing and allowed a unique structure to be assigned to it. The principle fragments whose elementary composition were measured by high resolution mass spectrometry (Table 1) are described in Scheme 1.

Table 1. High resolution mass spectrometry of sativanine-K

Peak	Formula	Div.	Found	Intensity (%)
$[M]^{\cdot+}$	$C_{27}H_{38}N_4O_6$	−1.4	514.2778	27.88
$[M-CO]^{\cdot+}$	$C_{26}H_{38}N_4O_5$	—	486	5.1
f	$C_{21}H_{27}N_3O_5$	+0.4	401.1954	0.6
g	$C_{21}H_{26}N_3O_5$	—	400	<0.5
h	$C_{20}H_{28}N_3O_4$	−2.6	374.2053	0.6
i	$C_{20}H_{27}N_3O_4$	0.5	373.2007	1.76
j	$C_{20}H_{26}N_3O_4$	−2.7	372.1897	0.88
p	$C_{11}H_{17}N_2O_2$	−0.8	209.1282	0.82
q	$C_{10}H_{17}N_2O$	—	181	<0.5
r	$C_{14}H_{15}N_2O_3$	−0.3	259.1080	0.64
s	$C_{13}H_{17}N_2O_2$	−1.3	233.1277	0.56
t	$C_{13}H_{14}NO_2$	−0.4	216.1020	2.03
	$C_9H_{11}NO_2$	+0.1	165.0791	80.1
	$C_7H_{12}NO_2$	−0.3	142.0865	100
	$C_6H_{12}NO$	+0.1	114.0920	92.1
	$C_5H_{11}NO_2$	+0.3	96.0453	1.9
	C_5H_9NO	+0.9	6.0978	36.2
	C_4H_6N	+0.7	68.0507	20.8

The various fragments have been named as described earlier [5].



Scheme 1. Mass spectrometric fragmentation of sativanine-K (1).

The mass spectrum of this new cyclopeptide alkaloid which carries a *N*-formyl group on the terminal amino acid, shows an intense [M^+] and the usual α-cleavage products are absent. The base peak is produced by the cleavage of the peptide bond between *N*-formyl isoleucine and hydroxyproline of the 13-membered ring system yielding the ion *m/z* 142 which then eliminates 2 × CO to give *m/z* 114 and *m/z* 86. The linkage and composition of the side chain with proline can be deduced by the fragment at *m/z* 142 ($C_7H_{12}NO_2$) forming the base peak. The attachment of hydroxyproline to *O*-methoxy-*p*-hydroxystyrylamine by an ether linkage is shown by the fragments **r**, **s**, **t** and also to isoleucine by an amide bonding on the other side, can be deduced by the peaks **p** and **q**. The fragment ions **f**, **g**, **h**, **i** and **j** represent the whole macrocyclic ring system of the molecule. In the acid

hydrolysate of **1** only isoleucine was confirmed by PC, thus confirming structure **1** for sativanine-K. Sativanine-K provides the first example of a naturally occurring *N*-formyl cyclopeptide alkaloid carrying a short side chain. In order to ensure that **1** was not an artefact produced during extraction with methanol, another sample of the bark was extracted with benzene–ammonia–EtOH and **1** was again isolated which proves that it is a naturally occurring compound [5].

EXPERIMENTAL

MS were measured at 70 eV with evapn of the sample in the ion source at *ca* 200°. TLC was carried out on silica gel HF_{254} and for PC Whatman No. 1 papers were used.

Extraction. Crude alkaloids (6.6 g) were obtained from the powdered bark (10 kg) as described earlier [7]. Another 5 kg plant material was extracted with C_6H_6–NH_3–EtOH (100:1:1) [8]. Compound (**1**) was also isolated using this method. The crude alkaloid mixture was fractionated on a 900 g silica gel column, eluting with increasingly polar CH_2Cl_2–MeOH mixtures into 15 fractions. The chromatographic sepn was monitored by UV and the collected fractions were analysed by TLC, proving in every case to be a mixture of two or three main components. The fractions were sepd into pure components using different chromatographic methods.

Sativanine-K (**1**). $C_{27}H_{38}N_4O_6$ ([M]$^+$ found 514.2778; calcd. 514.2792) 2.7 mg was obtained from fraction 12 [9] by TLC and chromatotron using $CHCl_3$–MeOH (20:1) and cyclohexane–EtOAc–MeOH (25:10:1). Mp 160–162°. UV λ_{max}^{MeOH} nm: 320 and 260 nm; IR γ_{max} cm^{-1}: 3370, 1665, 1630 (sec. amide), 2995–2830 (CH), 2820 (OMe), 1610 (C=C), 1220 + 1020 (aryl ether); MS: *m/z* 514 [M]$^+$, 486 [M − CO]$^+$, 457, 401, 400, 374, 373, 372, 259, 233, 209, 216, 181, 165, 142 (base peak), 114, 96, 86 and 68. **1** (2 mg) was hydrolysed with 6 N HCl (24 hr) in a sealed tube. The hydrolysate was evapd to dryness and examined by PC (*n*-BuOH–HOAc–H_2O, 4:1:5) [10] and (*n*-BuOH–benzyl alcohol–borate buffer pH 8.4, 45:45:8) [11] using ninhydrin as spray reagent. Isoleucine was identified by comparison with the authentic compound.

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